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Time to Positivity of a Rapid Bedside Assay for Cardiac-Specific Troponin T Predicts Prognosis in Acute Coronary Syndromes: A Thrombolysis in Myocardial Infarction (TIMI) 11A Substudy

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Objectives. **We sought to determine whether the rapid bedside assay for troponin T identified patients at risk for a more complicated hospital stay and a higher rate of adverse clinical events.**

Background. **In patients with an acute coronary syndrome, the amount of cardiac-specific troponin T released bears a stoichiometric relation to the extent of myocardial damage.**

Methods. **In 597 patients with unstable angina or non–Q wave myocardial infarction participating in the Thrombolysis in Myocardial Infarction (TIMI) 11A substudy, a rapid bedside assay and simultaneous quantitative serum measurement for troponin T were obtained at enrollment.**

Results. **The composite end point of the sum of death, nonfatal myocardial infarction or recurrent ischemia through day 14 occurred in 33.6% of patients with a positive assay compared with only 22.5% of patients with a negative assay ($p = 0.01$). Those patients in whom the rapid assay became positive in ≤10 min had the highest mortality rate of 4.2% through day 14 compared with 1.1% in those patients who had either a late-appearing positive assay (>10 min) or a negative assay. The duration of hospital stay in the 116 patients (19%) with a positive rapid assay at enrollment was a median of 5 days compared with only 3 days in the 481 patients (81%) with a negative rapid assay at enrollment ($p = 0.002$).**

Conclusions. **A positive rapid assay for troponin T at presentation identifies those patients at risk for higher rates of adverse clinical events and longer, more complicated hospital stays. Stratification of patients by time to development of a positive rapid assay identifies those patients at highest mortality risk.**

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Several studies have shown that patients with unstable angina or non–Q wave myocardial infarction (NQMI) are a heterogeneous group with a wide range of risks of adverse clinical outcomes (1,2). For example, patients >65 years of age are more than twice as likely to experience death or nonfatal myocardial infarction (MI) within 6 weeks of presentation compared with patients <65 years of age (2). The extent of myocardial necrosis sustained at the time of presentation with an acute coronary syndrome is an important determinant of the risk of adverse clinical outcome (3,4). The classic serum cardiac markers of myocardial damage, creatine kinase and its MB isoenzyme (CK-MB), suffer from a lack of specificity as well as a lack of sufficient sensitivity for detection of small amounts of myocardial necrosis (i.e., microinfarction) (5,6). In contrast, cardiac-specific troponin T (cTnT) has a greater specificity for myocardial necrosis than CK-MB and is also more sensitive to lesser degrees of myocardial necrosis (7–9). The amount of cTnT released into the blood stream bears a close stoichiometric relation to the amount of myocardial damage (10).

Detection of serum cTnT levels above the reference interval, using a commercially available quantitative enzyme immunoassay, identifies a subgroup of patients at increased risk of adverse clinical outcomes (11). The development by Katus and co-workers of a hand-held, rapid bedside assay enables clinicians to measure cTnT without the inherent delay of transporting specimens to a central chemistry laboratory (12). Previous evaluations of the rapid assay focused predominantly on its ability to diagnose MI when studied in a single, dedicated research center (12). The objectives of this study were 1) to determine if the rapid assay for cTnT could identify patients with unstable angina or NQMI who are at risk for a higher rate of adverse clinical events and a more complicated hospital stay; 2) to determine whether the time to positivity of the assay could be used as an alternative method for estimating cTnT measurements and would be predictive of mortality risk; and 3)

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Abbreviations and Acronyms

CABG = coronary artery bypass graft surgery
CK-MB = creatine kinase, MB isoenzyme
cTnT = cardiac-specific troponin T
ECG = electrocardiogram
MI = myocardial infarction
NQMI = non–Q wave myocardial infarction
TIMI = Thrombolysis in Myocardial Infarction

to determine whether the rapid assay was a useful bedside tool that could be used in a widespread field application across multiple clinical centers.

Methods

We evaluated the prognostic potential of the Cardiac T Rapid Assay (Boehringer Mannheim Corp.) in the 45 centers participating in the previously reported Thrombolysis in Myocardial Infarction (TIMI) 11A trial (13). This was an open-label, dose-ranging study of the low molecular weight heparin preparation enoxaparin in patients with unstable angina and NQMI. Patients were eligible for enrollment in TIMI 11A if a qualifying episode of ischemic discomfort occurred within the preceding week and was characterized by either rest angina lasting ≥5 min, new-onset angina or increased severity of a previously stable anginal pattern. Major exclusion criteria included an evolving Q wave MI, coronary artery bypass graft surgery (CABG) within the previous 2 months and contraindications to anticoagulation (13). Patients were considered to have had a NQMI at enrollment if CK-MB measurements at the enrolling site were above normal (and ≥3% of total CK) at baseline or ~8 h after enrollment, or if CK-MB measurements were elevated at the 16-h sample and no ischemic discomfort for ≥30 min occurred between enrollment and the 16-h sample.

The cTnT substudy protocol specified that a rapid assay should be performed at enrollment along with a simultaneously drawn serum specimen for quantitative assessment of cTnT. Clinical events assessed in the group undergoing cTnT testing included 1) death (all-cause mortality); 2) recurrent nonfatal MI (defined by [re]elevation of CK-MB levels above normal [fivefold elevation after CABG] and/or new Q waves or left bundle branch block on the electrocardiogram [ECG]); and 3) recurrent ischemic discomfort. The composite end point noted previously was selected for the cTnT substudy based on previous observations that patients with unstable angina or NQMI presenting with elevated cTnT levels are at increased risk for multiple adverse clinical events, including mortality and recurrent infarction, and also undergo revascularization procedures more frequently (11). This suggests that patients with elevated cTnT at presentation have sufficient coronary artery disease to place them at risk not only for an initial episode of myocardial necrosis but also for repeated bouts of myocardial ischemia and infarction.

Rapid assay for cTnT. The test is performed by placing 150 μl of whole blood in the specimen well, where cTnT reacts with two monoclonal antibodies (12,14). The cTnT in the patient's blood binds to the two antibodies and forms a sandwich that flows along a glass-fiber fleece toward the read zone. The immune complexes are immobilized in the read zone by an interaction between streptavidin (bound to the cellulose nitrate membrane) and the biotinylated antibody. This ultimately leads to production of a purplish-red line in the read zone. The intensity of the color and the speed with which it develops correlate with the concentration of cTnT in the patient's blood (12). Another red line develops in an on-board control area where unreacted gold-labeled antibodies react with cTnT bound to the membrane. The rapid assay is considered positive if by 20 min two red lines form, even if the patient line appears only faintly. The detection limit of this version of the rapid assay is 0.2 ng/ml of cTnT.

The protocol specified that the rapid assay be read locally by clinical personnel not directly involved in the care of patients enrolled in TIMI 11A and the results not be used in clinical decision-making. The test was read as either positive or negative. Positive results were categorized as to whether the red line in the patient area appeared within ≤10 min or >10 min. Patients who had a negative rapid assay were categorized as to whether they presented ≤6 h or >6 h from the onset of ischemic discomfort.

Quantitative assay for cTnT. Blood specimens were drawn at presentation (simultaneous with rapid assay) and centrifuged locally, and the serum was extracted. Samples were stored at −20°C until shipment to the core laboratory, where they were analyzed for cTnT on the ES-300 immunoanalyzer using the Enzymun assay. This assay uses complementary dual cardiac-specific monoclonal antibodies and has a detection limit of 0.01 ng/ml (15).

Statistical analysis. The results of the rapid assay and quantitative assay for cTnT were merged with the clinical data base for TIMI 11A at the Data Coordinating Center for the main trial (Covance, Princeton, New Jersey), where the analyses were performed. With regard to baseline characteristics and clinical outcomes, patients who had a positive rapid assay were compared with patients who had a negative rapid assay, using the Student *t* test for continuous variables and the chi-square test for dichotomous variables. Plots depicting time to clinical events were constructed using the Kaplan-Meier method, and curves were compared using the log-rank test. The quantitative cTnT results in various patient groups stratified by the results of the rapid assay were compared using the Kruskal-Wallis test. All statistical comparisons were two-tailed, and $p < 0.05$ was considered statistically significant.

Results

Baseline characteristics. Of the 630 patients enrolled in TIMI 11A between July 1995 and January 1996, 597 (95%) had rapid assays performed and participated in the cTnT substudy. Of the 597 patients with rapid assays performed at enrollment,

Table 1. Baseline Characteristics

	Troponin T Substudy (n = 597)		
	Positive Rapid Assay by 20 min (n = 116)	Negative Rapid Assay (n = 481)	Main TIMI 11A Trial (n = 630)
Age (yr)	65 (55, 71)	64 (54, 71)	64 (54, 71)
Gender			
Male	80 (69)	298 (62)	399 (63)
Female	36 (31)	183 (38)	231 (37)
Race			
White	100 (86)	384 (80)	512 (81)
Other	16 (14)	97 (20)	118 (19)
Diabetes	39 (34)	157 (33)	203 (32)
Current smoker	34 (29)	122 (26)	163 (26)
Hypertension	74 (64)	287 (60)	375 (60)
Previous cardiac history			
Angina	74 (64)	351 (73)	445 (71)
Previous angiogram with ≥50% stenosis	55 (48)	275 (59)	348 (55)
PTCA	24 (21)	157 (33)	194 (31)
CABG	28 (24)	122 (25)	158 (25)
MI	52 (45)	175 (37)	242 (38)
Medications before enrollment			
ASA	102 (88)	383 (80)	512 (81)
IV nitrates	56 (48)	98 (20)	160 (25)
Beta-blocker	62 (53)	230 (48)	304 (48)
IV heparin	80 (69)	195 (41)	286 (45)
Characteristics of presenting illness			
Time since qualifying pain (h)	32 (14, 62)	14 (6, 31)*	16 (7, 38)
Non–Q wave MI (CK-MB criteria)	19 (16)	28 (6)*	48 (8)

*$p < 0.001$ for comparison between groups with a positive versus negative rapid assay. For continuous variables the values shown indicate the median (25th, 75th percentiles); for dichotomous variables the values indicate the number (%) of patients with a given finding. ASA = acetylsalicyclic acid (aspirin); CABG = coronary artery bypass graft surgery; CK-MB = creatine kinase, MB isoenzyme; IV = intravenous; MI = myocardial infarction; PTCA = percutaneous transluminal coronary angioplasty.

538 (90%) had a simultaneous quantitative measurement of cTnT in the core laboratory. The rapid assay at enrollment was positive by 20 min in 116 patients and by 10 min in 48 patients, and was negative in 481 patients. The two groups of patients stratified by a positive or negative rapid assay by 20 min at enrollment had similar baseline characteristics and were also representative of the entire group enrolled in the TIMI 11A trial (Table 1). However, the interval between the onset of ischemic discomfort and performance of the rapid assay was significantly shorter in patients with a negative assay ($p < 0.001$).

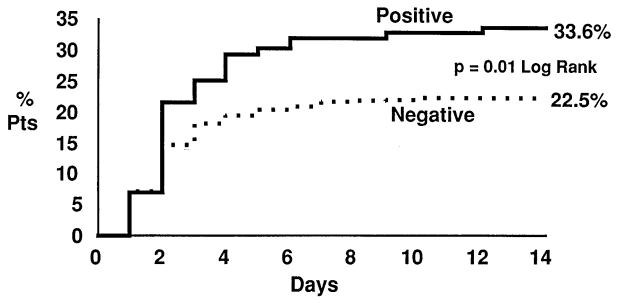
Clinical events. The composite end point of the sum of death, nonfatal MI or recurrent ischemia through day 14 occurred in 33.6% of patients with a positive assay compared with 22.5% of patients with a negative assay ($p = 0.01$ by the chi-square test). Kaplan-Meier plots of the time to the first of any of the events noted earlier revealed an early separation by 3 days and a persistent separation of the curves by day 14 ($p = 0.01$ by the log-rank test) (Fig. 1).

The duration of hospital stay was a median of 5 days (interquartile range 3 to 8) in patients with a positive rapid assay, but 3 days (interquartile range 2 to 5) in patients with a

Figure 1. Kaplan-Meier plot of time to first adverse clinical event (death, nonfatal MI or recurrent ischemia) through day 14 in patients (Pts) with a positive or a negative rapid assay for cTnT. The distribution of clinical events in the two groups is shown in the tabulation below:

Event	Pos (n = 116)	Neg (n = 481)
Death	2 (1.7%)	7 (1.5%)
MI	2 (1.7%)	8 (1.7%)
Rec isch	35 (30.2%)	93 (19.3%)
Any	39 (33.6%)	108 (22.5%)

Neg = negative rapid assay; Pos = positive rapid assay; Rec isch = recurrent ischemia.



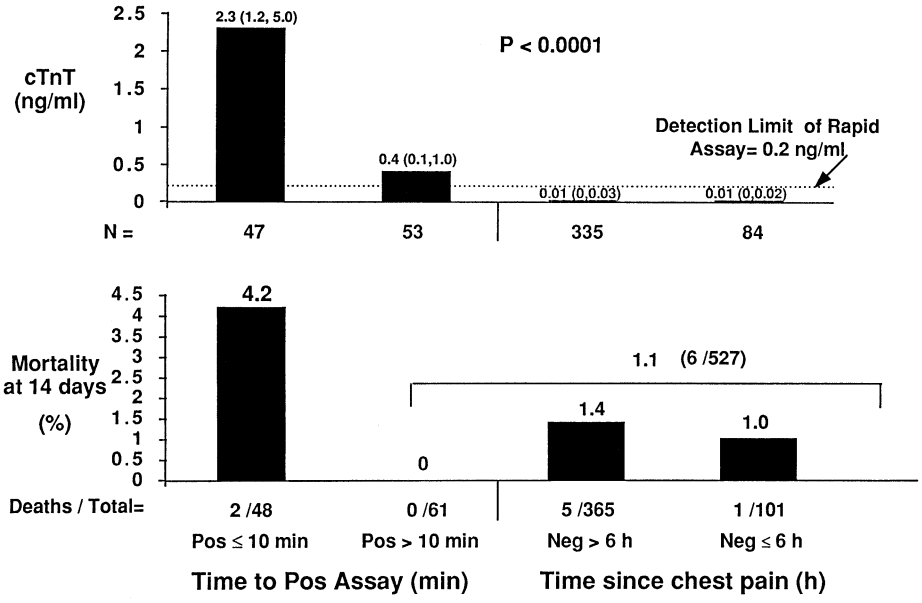


Figure 2. Quantitative cTnT and mortality findings stratified by results of rapid assay at study enrollment. In both the top and bottom graphs, four groups of patients are shown: positive (Pos) rapid assay ≤10 min; positive rapid assay >10 min; negative (Neg) rapid assay but presentation >6 h from onset of discomfort; and negative rapid assay but presentation ≤6 h from onset of ischemic discomfort. **Top,** Simultaneously measured quantitative serum cTnT levels (median [25, 75 percentiles]) were highest in patients with an early positive rapid assay and were progressively lower in the other patient groups depicted. $p < 0.0001$ by the Kruskal-Wallis test. **Bottom,** Mortality through 14 days was highest in the patients with an early positive rapid assay compared with the patients with a late positive rapid assay or negative rapid assay at enrollment. Note: The total number of patients in each of the four groups is different in the top and bottom graphs because not all patients with a rapid assay had a simultaneous quantitative cTnT measurement.

negative rapid assay at enrollment ($p = 0.002$). The composite end point of death, nonfatal MI, recurrent ischemia and the performance of percutaneous transluminal coronary angioplasty or CABG through hospital discharge occurred in 50.9% of patients with a positive rapid assay at enrollment, but in 37.8% of patients with a negative rapid assay ($p = 0.01$).

Correlation of rapid assay with quantitative cTnT and mortality. Among the 538 patients with both a quantitative assessment and rapid assay for cTnT at enrollment, 110 (20%) and 428 (80%) had quantitative cTnT values ≥0.2 ng/ml and <0.2 ng/ml, respectively. The rapid assay for cTnT was positive by 20 min in 107 (20%) and negative in 431 (80%) patients. Of the 431 patients with a negative rapid assay, 24 (6%) had a quantitative cTnT value ≥0.2 ng/ml (median 0.31 [range 0.23 to 0.43]). A rapid assay by 20 min had a sensitivity of 78% and a specificity of 95% for detection of a quantitative cTnT value ≥0.2 ng/ml.

As depicted in Figure 2A, those patients who had a positive rapid assay in ≤10 min had a median cTnT level of 2.3 ng/ml(range 1.2 to 5.0) compared with 0.4 ng/ml (range 0.1 to 1.0) when the assay was positive in >10 min. Patients who had a negative rapid assay but who presented both >6 h from the onset of chest pain and ≤6 h from the onset of chest pain had median cTnT levels of 0.01 ng/ml. The differences in the cTnT levels among the four groups of patients shown in Figure 2A were significant at $p < 0.001$.

The mortality rate through 14 days in the patients with a quantitative cTnT level ≥0.2 ng/ml was 2.7% compared with 1.2% in patients with a quantitative cTnT level <0.2 ng/ml. However, those patients who had a positive rapid assay in ≤10 min had the highest mortality rate of 4.2% through day 14 (Fig. 2B). There were no deaths in the group with a positive assay in >10 min. The mortality rate in the patients with a negative assay who presented >6 h from the onset of pain was 1.4% compared with 1.0% in patients with a negative assay presenting within 6 h of pain. The mortality rate in those patients who had either a late-appearing positive rapid assay (>10 min) or a negative assay, collectively, was 1.1%.

Comparison with CK-MB. Of the total of 597 patients participating in the cTnT substudy, 47 (8%) were diagnosed as having a NQMI at enrollment by CK-MB criteria as compared with 116 (19%) who had a positive rapid assay for cTnT. The 14-day mortality rate was 2.1% in the patients diagnosed as having NQMI by CK-MB compared with 1.7% in those with a positive rapid assay by 20 min and 4.2% in those with a positive rapid assay in ≤10 min.

Discussion

Previous studies in patients with unstable angina or NQMI, using quantitative measurements of cardiac-specific troponins in specialized core laboratories, have shown a gradient of risk for adverse clinical events with detection of increasing quantities of these proteins in the blood at the time of clinical presentation (11,16). The prognostic information conveyed by

measurement of the cardiac troponins was independent of demographic characteristics and ECG findings (11,16).

Our study demonstrates that patients with a positive rapid assay are at higher risk of adverse clinical events and longer, more complicated hospital stays. By using the speed of color development of the red line in the read zone of the rapid assay, it is possible to arrive at a semiquantitative estimate of the concentration of cTnT in a patient's blood. Importantly, our findings show for the first time that the time to positivity of the rapid assay is predictive of mortality risk. Prognostication using the time to positivity of the rapid assay compared favorably with simple dichotomization of quantitative cTnT measurements at a cutoff level of 0.2 ng/ml, as well as diagnosis of NQMI by conventional CK-MB criteria, with 14-day mortality rates tending to be highest in those with an early (≤10 min) positive rapid assay. The absolute mortality rates and the gradient of mortality risk depicted in Figure 2B are nearly identical to those reported by the Fragmin During Instability in Coronary Artery Disease (FRISC) investigators who stratified a group of patients with unstable angina according to quantitative cTnT measurements in a core laboratory (17). The data contained in the FRISC report show a 14-day mortality rate of ~4% in patients with the highest quintile of cTnT (≥2.12 ng/ml) and ≤1% in patients with the lowest quintiles of cTnT (<0.62 ng/ml). Finally, our evaluation of the rapid assay has demonstrated it to be a useful prognostic tool when tested in a widespread field application in 45 separate centers.

Clinical implications. The rapid assay is a reliable, convenient alternative method for bedside assessment of the level of cTnT in a patient's blood at the time of presentation with an acute coronary syndrome. As shown in our report, not only does the rapid assay extend the capability of measuring cTnT to the site where medical care is being delivered, but it also provides clinicians with a tool for prognostication at the patient's bedside. The data in Figure 2 indicate that the rapid assay for cTnT conveys the same diagnostic and prognostic information provided by a quantitative test for cTnT. In the context of other reports documenting the diagnostic and prognostic efficiency of the rapid assay in patients with ST segment elevation, our study extends the evidence that the rapid assay for cTnT is useful across the entire spectrum of acute coronary syndromes, and may be helpful to clinicians when assigning a patient to an intensive care setting versus a stepdown unit or another less costly facility (14,18,19). Of note, negative rapid assays must be analyzed with reference to the clinical circumstances under which they were obtained. As with all assays of serum cardiac markers, the rapid assay for cTnT should be interpreted in the context of the number of hours elapsed from the onset of ischemic discomfort (14). Because of the kinetics of cTnT release from the myocardium, patients presenting <6 h from the onset of symptoms may have a negative rapid assay (Table 1), yet be at risk for adverse clinical events (Fig. 2B).

Use of the rapid assay for cTnT eliminates the delay in turnaround time, even in hospitals with chemistry laboratories offering quantitative cTnT measurements on an urgent basis. It also provides clinicians with a simple means for measuring cTnT, even if a quantitative test is not available in their hospital, and makes such assessment possible in other settings such as the physician's office, free-standing clinic or ambulance. Technologic improvements, including subsequent generations of the rapid assay with an even lower detection limit (0.08 ng/ml) and which can be read within 15 min, are anticipated to increase the clinical appeal of this device even further.

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